

TNTplus Method
Method 10276
0.015 to 0.500 mg/kg VDK
TNTplus™ 819

Scope and application: For beer. The analysis follows the chemistry in *MEBAK Wort, Beer, Beer-Based Beverages*, Edition 1, 2012.



Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for TNTplus vials

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800	—	LZV646

Before starting

DR 3900, DR 3800: Install the light shield in Cell Compartment #2 before this test is started.

Review the safety information and the expiration date on the package.

The recommended temperature for reagent storage is 2–8 °C (35–46 °F).

Keep the DosiCap Zip in dark areas when not in use.

Distill the sample in agreement with MEBAK or ASBC regulations before the analysis is started.

This method is not applicable to the DR 1900 or the DR 2800 instrument.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

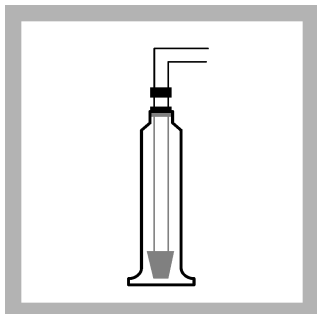
Description	Quantity
VDK TNTplus Reagent Set	1
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet tips, for 1.0–5.0 mL pipet	1
Distillation apparatus	1

Refer to [Consumables and replacement items](#) on page 4 for order information.

Sample collection

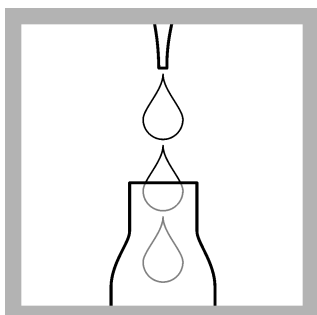
- Collect samples in clean glass bottles.
- Analyze the samples as soon as possible for best results.

Distillation

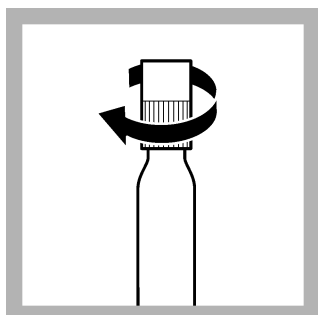


1. Use MEBAK or ASBC analysis methods to distill 100 g of uncarbonated beer to 25 mL of sample distillate. Use the sample distillate in the test procedure.

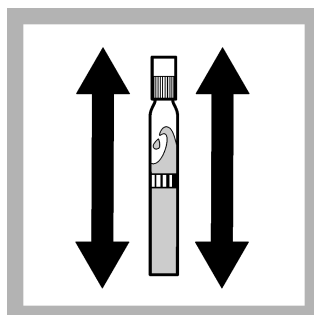
Test procedure



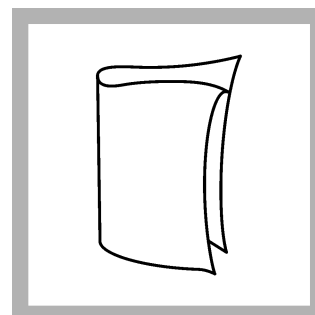
1. Use a pipet to add 4.0 mL of distillate to the test vial.



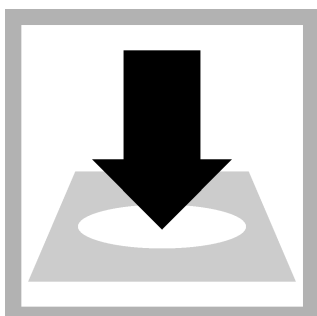
2. Tighten the DosiCap Zip on the vial.



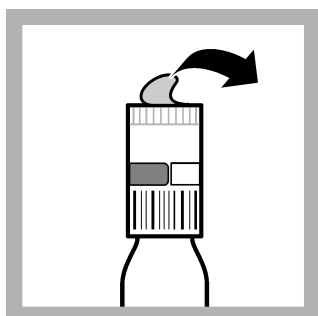
3. Shake vigorously 10 times to mix.



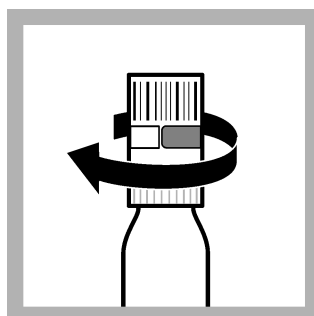
4. Clean the vial.



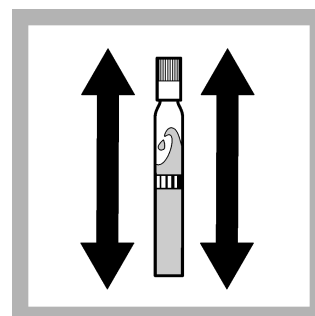
5. Insert the vial into the cell holder. The instrument reads the barcode and shows E1 on the display. The instrument zero is set.



6. Carefully remove the lid from the DosiCap™ Zip cap. Remove the cap from the test vial.



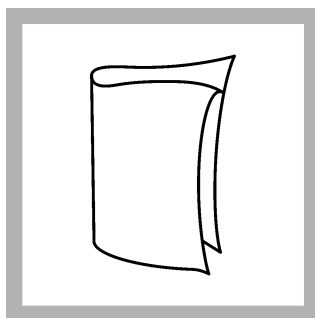
7. Turn the DosiCap Zip over so that the reagent side goes on the test vial. Tighten the cap on the vial.



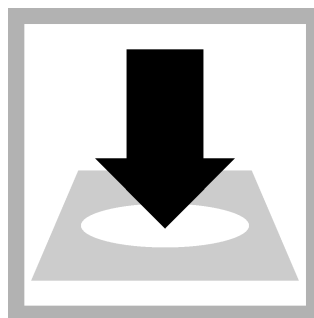
8. Shake the vial vigorously to dissolve the reagent in the cap. Look through the open end of the DosiCap to make sure that the reagent has dissolved.



9. Start the reaction time of 5 minutes.



10. When the timer expires, clean the vial.



11. Insert the vial into the cell holder. Results show in mg/kg VDK.

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Note: The distillation procedure is not necessary for the check standards because the standards are already VDK and not beer. Since the standards are not distilled, the concentration of the check standard is 25% of the nominal concentration. During the distillation procedure 100 mL of beer is distilled down to 25 mL, which makes the distillate four times more concentrated. Thus, to prepare a 0.5-mg/kg standard, it is necessary to prepare a 2.0-mg/kg standard to have the check standard read 0.5-mg/kg with the stored program.

Items to collect:

- 2,3-Butanedione, 99% solution
 - 250-mL volumetric flask, Class A
 - Pipet, adjustable volume, 100–1000 μ L and pipet tip
 - Pipet, adjustable volume, 1.0–5.0 mL and pipet tip
 - Deionized water
1. Prepare a 500-mg/kg VDK¹ stock solution as follows:
 - a. Use a pipet to add 126 μ L of 2,3-Butanedione solution into the volumetric flask.
 - b. Dilute to the mark with deionized water. Mix well. Keep the solution in an amber bottle in the refrigerator.
 2. Prepare a 0.5-mg/kg VDK² standard solution as follows:
 - a. Use a pipet to add 1 mL of the 500-mg/kg VDK stock solution into a 250-mL volumetric flask.
 - b. Dilute to the mark with deionized water. Mix well. Prepare this solution weekly.
 3. Use the test procedure to measure the concentration of the prepared standard solution.
 4. Compare the expected result to the actual result.

Summary of method

The vicinal diketones diacetyl (2,3-Butanedione) and 2,3-Pentanedione that are formed by the yeast metabolism react with o-Phenylenediamine (OPD) to form 2,3-Dimethylquinoxaline, which is measured photometrically at 335 nm.

¹ This solution is a 500-mg/kg VDK standard.

² This standard is a nominal 2.0-mg/kg standard. Refer to the note above.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
VDK TNTplus Reagent Set	1	25/pkg	TNT819

Required apparatus

Description	Quantity/test	Unit	Item no.
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Light shield, DR 3800	1	each	LZV646
Light shield, DR 3900	1	each	LZV849

Recommended standards

Description	Unit	Item no.
2,3-Butanedione, 99%, 100 g	1	2446732

Optional reagents and apparatus

Description	Unit	Item no.
Flask, volumetric, Class A, 250 mL	each	2636646
Pipet, adjustable volume, 1.0–5.0 mL	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	75/pkg	BBP068
Pipet, Tensette®, 0.1-1.0 mL	each	1970002
Pipet tips, for 100-1000 µL pipet	1000/pkg	2795000
Water, deionized	4 L	27256



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